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Apomyoglobin forms a micellar complex with phospholipid at low pH

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Apomyoglobin was found to break down phosphatidylcholine vesicles to form a micellar complex at pH 4. Size reduction during this process was observed by measuring light scattering at 400 nm and by passing the complex through a gel-filtration column. Micellar complex formation was confirmed by electron microscopy.

Apomyoglobin; Micelle formation; Amphiphilic α-helix; Phosphatidylcholine; Lipid bilayer; Discoidal structure

1. INTRODUCTION

Proteins and phospholipids are the major constituents of biological membranes. Study of the nature of the interaction between these two classes of molecules is important for understanding the structure and function of biological membranes. One of the most unusual but fascinating modes of interaction between lipid bilayers and proteins is the breakdown of vesicles by proteins forming an overall micellar configuration but still retaining the phospholipid bilayer structure [1–5]. Although the detailed mode of interaction between phospholipids and proteins in these micellar complexes is yet to be worked out, it has been suggested that amphiphilic α-helix segments of the protein surround the edge of a lipid bilayer patch fragmented from the vesicle forming a 'discoidal' structure [6]. The hydrophobic surface of the α-helix interacts with the hydrophobic tail part of the phospholipid while the hydrophilic surface of the α-helix faces the aqueous environment, thus preventing direct contact of the lipid acyl chains with water.

Since myoglobin (Mb) consists mainly of α-helix and these helices are predominantly amphiphilic [7], it occurred to us that the apoprotein may form a micellar complex with phospholipid under appropriate conditions. We found that this is the case at low pH. Earlier, it was observed that a segment of apomyoglobin (apoMb) penetrates the hydrophobic interior of PC/PS vesicles in acidic media (Choi, Y.H. and Kim, H., unpublished).

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Abbreviations: Mb, myoglobin; apoMb, apomyoglobin; PC, phosphatidylcholine; PS, phosphatidylserine; DMPC, dimyristoylphosphatidylcholine; *L/P*, lipid/protein molar ratio; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; TLC, thin-layer chromatography

2. MATERIALS AND METHODS

Mb from sperm whale was obtained from Sigma and the heme was removed by the 2-butanone extraction procedure [8]. The possible contamination of the apoprotein by Mb was assessed spectrophotometrically [9]. In all cases no significant absorption was observed in the Soret region. Also, the homogeneity of the preparations was checked by SDS-PAGE using a modified Laemmli discontinuous buffer system [10]. DMPC was purchased from Sigma and its purity was confirmed by TLC.

ApoMb concentration in the absence of phospholipid was determined spectrophotometrically at 280 nm [9]. In the presence of phospholipids, it was determined by the modified Lowry method of Markwell et al. [11] with Mb as a standard.

Light-scattering measurements of DMPC/apoMb mixtures at various *L/P* molar ratios were performed with a Jasco FP-770

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spectrofluorometer (400 nm, 23°C) at pH 4 (0.01 M acetate buffer, 0.1 M NaCl). The same samples were incubated for 1 h and the DMPC/apoMb mixture was passed through a Sepharose CL-4B column (1.8 × 38 cm). Another batch of mixtures was negatively stained with 2% phosphotungstate and characterized with a Jeol JEM-100CX II electron microscope. Quenching of fluorescence by iodide was observed with the spectrofluorometer using identical conditions to light-scattering measurements, except for excitation at 280 nm.

3. RESULTS AND DISCUSSION

The changes in light scattering of the vesicle suspension with time at pH 4 after the injection of apoMb solution are presented in fig.1. The DMPC/apoMb ratio was varied by changing the apoMb concentration while fixing the amount of sonicated DMPC vesicles (0.5 mM). The light scattering increased transiently and then decreased to a value lower than the initial value. This may suggest that the DMPC vesicles pass through an initially aggregated state then fragment into smaller micellar complexes. At an L/P value of 600, the intensity of the scattered light did not drop below the value of pure vesicles. However, at an L/P value of less than 60, the scattering intensity decreased to the minimum plateau value within 8 min after the mixing. There was no change in light scattering when apoMb was added to the DMPC vesicles at pH 7 (not shown).

It is clear that the vesicles were broken down into smaller units within about 10 min after the initial aggregation when the L/P value is less than 100. This process is much faster than the case of micellar complex formation by apolipoprotein A-I protein [12].

Fig.2 compares the elution profile of DMPC/apoMb mixture when L/P was 80 with those of pure vesicles and pure apoMb in a Sepharose CL-4B column. A protein/lipid complex (elution volume 60 ml) with a size between vesicles and protein was formed, demonstrating again that the vesicles were broken down into smaller units by apoMb.

The formation of a micellar complex of DMPC and apoMb can be clearly demonstrated by the electron microscope as shown in fig.3B. Disks of about 32 nm diameter and a thickness of 4 nm can be seen. This may be compared with the control electron micrograph of the vesicles shown in fig.3A. This electron micrograph picture is strikingly similar to those of PC/apolipoprotein A-I protein complex [14]. The disk stacking-up was regarded as staining artifacts [1], rather than a real picture.

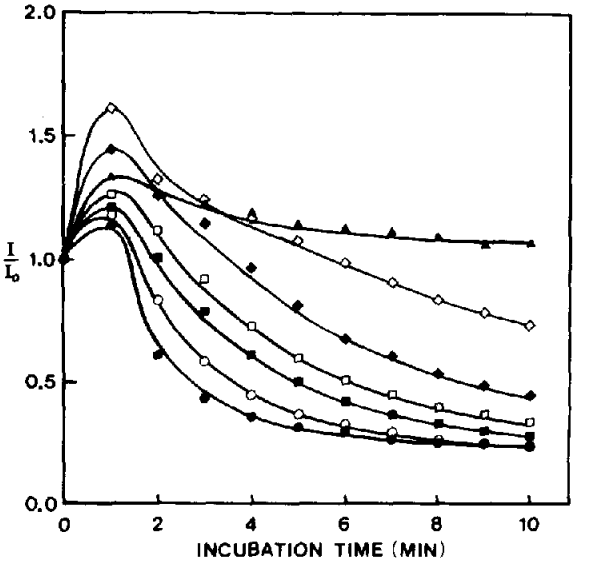

Fig.1. Light-scattering measurement as a function of time and L/P molar ratio (●—●, L/P 40; ○—○, L/P 60; ■—■, L/P 80; □—□, L/P 100; ◆—◆, L/P 160; ◇—◇, L/P 400; ▲—▲, L/P 600). I, intensity of scattered light; I_0, intensity of scattered light by vesicles alone. See text for details.

The experimental results presented here unequivocally demonstrate that the apoMb fragments DMPC vesicles to form a smaller micellar complex

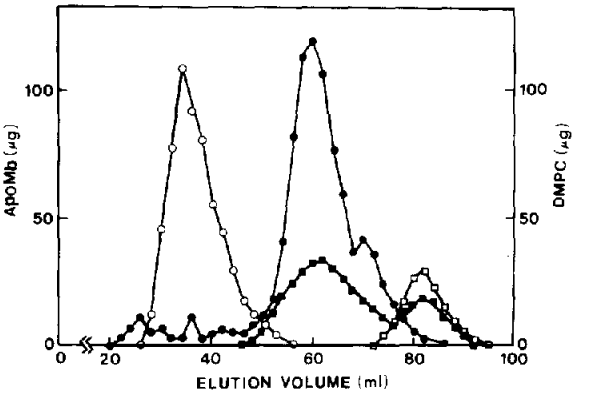

Fig.2. Gel filtration on Sepharose CL-4B. Sonicated DMPC vesicles (○—○) and free apoMb (□—□) are monitored by measuring the absorbance at 280 nm. The DMPC (●—●) in the micellar complex is determined by the method of Vaskovsky [13] and apoMb (■—■) by the method of Markwell et al. [11].

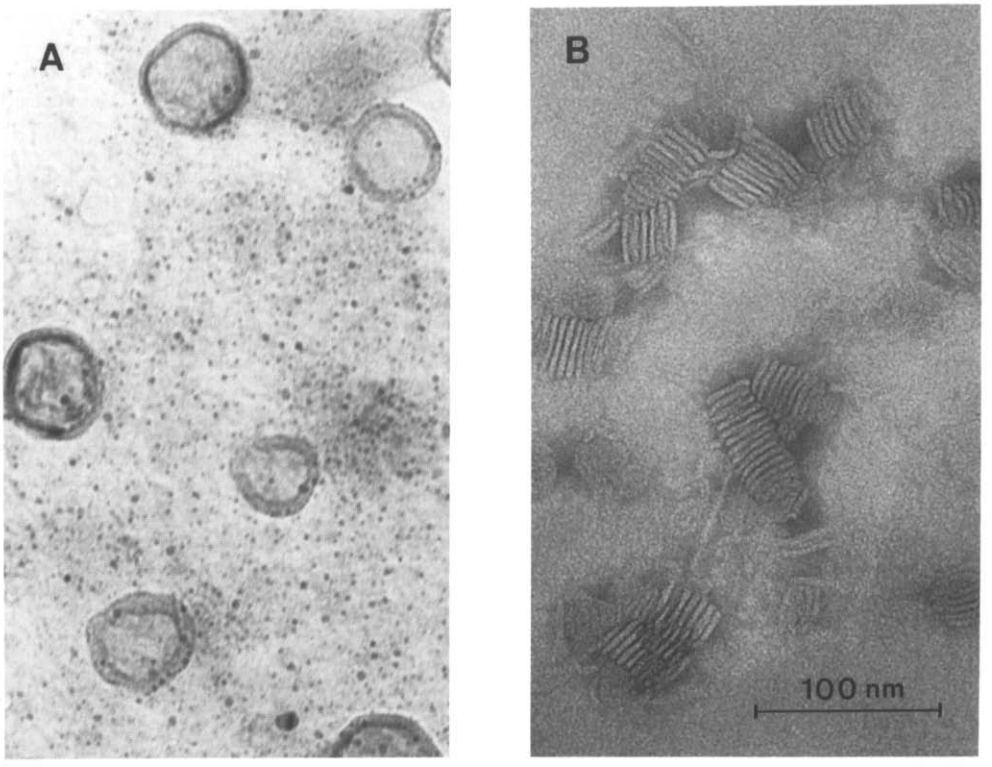

Fig.3. Negative stain electron microscopy of sonicated DMPC vesicles (A) and DMPC/apoMb complex at L/P 80 (B).

at low pH but not in the neutral pH range. Some differences between the apoMb structure at low pH and that at neutral pH apparently bring about different interaction modes. Conversion of Mb into its apoprotein causes a slight swelling and a reduction in α-helix content [15] but the general feature of the Mb structure is still intact. When the pH of the medium is lowered, there is a further decrease in α-helix content and the two tryptophan residues situated in the N-terminal region of the protein are somewhat more exposed to the solvent [15]. Still, the N-terminal region of the apoMb retains a considerable degree of secondary as well as tertiary structure while the rest of the protein loses all the structure [16,17]. The decrease in α-helix content was also observed for met-Mb at low pH. The secondary structure of the C-terminal region of the holoprotein is largely destroyed while the α-helix in the N-terminal region remains intact. The fluorescence quenching by iodide showed that the quenching is much less extensive in the presence of phospholipid vesicles at low pH (fig.4). This in-

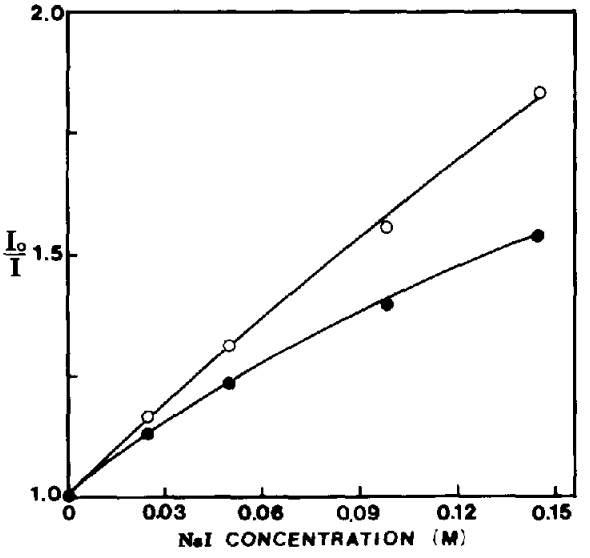

Fig.4. Quenching of the intrinsic fluorescence of apoMb by NaI in the absence (○—○) and presence (●—●) of DMPC (L/P = 40). I_0, fluorescence intensity before the addition of NaI; I, intensity after NaI addition. The incubation time for the micellization was 1.5 h.

dicates that at least the N-terminal region is in contact with the hydrophobic part of the micellar complex. In this regard, it is interesting that the 1–42 amino acid residues are protected from tryptic digestion when present together with PC/PS (4:1) vesicles at low pH (Choi, Y.H. and Kim, H., unpublished).

The important questions remaining to be resolved are whether the C-terminal regions are also involved in the formation of micellar complex, the structure and topology of the apoprotein within the complex, and the mechanism of complex formation. Shedding some light on these questions is the aim of our current investigation.

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